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Erosion Protection by Calcium Lactate/ Sodium Fluoride Rinses under Different Salivary Flows in vitro

Alessandra B. Borges[a, b] Taís Scaramucci[b] Frank Lippert[b] Domenick T. Zero[b] Anderson T. Hara[b]

[a]Department of Restorative Dentistry, Universidade Estadual Paulista, São José dos Campos, Brazil; [b]Oral Health Research Institute, Department of Preventive and Community Dentistry, Indiana University School of Dentistry, Indianapolis, Ind., USA

Key Words
Calcium · Dentin · Enamel · Erosion · Fluoride · Optical profilometry · Salivary flow rate

Abstract
This study investigated the effect of a calcium lactate prerinse on sodium fluoride protection in an in vitro erosion-remineralization model simulating two different salivary flow rates. Enamel and dentin specimens were randomly assigned to 6 groups (n = 8), according to the combination between rinse treatments – deionized water (DIW), 12 mM NaF (NaF) or 150 mM calcium lactate followed by NaF (CaL + NaF) – and unstimulated salivary flow rates – 0.5 or 0.05 ml/min – simulating normal and low salivary flow rates, respectively. The specimens were placed into custom-made devices, creating a sealed chamber on the specimen surface connected to a peristaltic pump. Citric acid was injected into the chamber for 2 min, followed by artificial saliva (0.5 or 0.05 ml/min) for 60 min. This cycle was repeated 4×/day for 3 days. Rinse treatments were performed daily 30 min after the 1st and 4th erosive challenges, for 1 min each time. Surface loss was determined by optical profilometry. KOH-soluble fluoride and structurally bound fluoride were determined in specimens at the end of the experiment. Data were analyzed by 2-way ANOVA and Tukey tests (α = 0.05). NaF and CaL + NaF exhibited significantly lower enamel and dentin loss than DIW, with no difference between them for normal flow conditions. The low salivary flow rate increased enamel and dentin loss, except for CaL + NaF, which presented overall higher KOH-soluble and structurally bound fluoride levels. The results suggest that the NaF rinse was able to reduce erosion progression. Although the CaL prerinse considerably increased F availability, it enhanced NaF protection against dentin erosion only under hyposalivatory conditions.

Sodium fluoride is a well-established agent for caries prevention, able to slow down or to reverse the carious process [Featherstone, 2008]. In dental erosion, the protective action of sodium fluoride is not fully elucidated. It has been speculated that it is attributed to the remineralization of previously eroded enamel, leading to the formation of a less soluble fluoridated apatite structure where hydroxyl groups of hydroxyapatite are replaced by fluoride ions [Parkinson et al., 2010]. It has also been suggested that CaF_2-like deposits on the tooth surface create an additional mineral layer to be dissolved by acid challenges, therefore protecting the subjacent enamel [Ganss et al., 2001, 2007]. Alternatively, fluoride can also interact with hydroxyapatite by adsorbing onto the enamel crys-

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Anderson T. Hara
Oral Health Research Institute, Department of Preventive and Community Dentistry
Indiana University School of Dentistry, 415 N. Lansing Street
Indianapolis, IN 46202-2876 (USA)
E-Mail ahara@iu.edu

tallite surface, inhibiting dissolution [Arends and Christoffersen, 1990]. Nevertheless, in in vitro studies, the sodium fluoride efficacy against erosion has been found to be limited, especially for enamel [Ganss et al., 2001].

Enamel remineralization is driven by the availability of fluoride, calcium and phosphate ions in the environment surrounding the demineralized dental surfaces [Cochrane et al., 2010]. Enhancement of sodium fluoride protection against erosion has been attempted by the use of highly fluoridated prescription products. Although sometimes a dose response can be observed, it can be speculated that sodium fluoride efficacy may reach a plateau at a certain concentration, beyond which it does not provide additional protection for particular in vitro erosive conditions [White et al., 2012]. Considering this potential limitation, fluoride association with a calcium prerinse has been proposed, in order to increase intraoral sites for fluoride retention [Vogel et al., 2006, 2008b]. A preliminary in situ study has suggested erosion protection action by the calcium lactate prerinse [Turssi et al., 2012].

The dynamic interaction between Ca and F ions is modulated by different salivary factors, including flow rate. Therefore, subjects with impaired salivary flow may be highly susceptible to dental erosion, as this condition reduces the individual ability to clear, dilute and neutralize the erosion-inducing acids [Featherstone and Lussi, 2006; Millward et al., 1997], modifying the intraoral availability and retention of Ca and F ions. Hyposalivation affects approximately 30% of patients aged between 20 and 69 years [Flink et al., 2008] and can be related to aging, diseases, radiation, medications, and specific dietary and exercise patterns [Tschoppe et al., 2010].

In this study, we hypothesized that a Ca prerinse followed by a sodium fluoride rinse would increase the retention of fluoride on enamel and dentin surfaces [Vogel et al., 2006], and this would consequently improve erosion protection particularly under hyposalivatory conditions. Therefore, the objective was to investigate the antierosive potential of a sodium fluoride rinse treatment associated or not with a calcium lactate prerinse, in an in vitro erosion model simulating two different salivary flow rates.

Materials and Methods

Study Design

This study consisted of a factorial 3 × 2, testing rinse treatments at 3 levels – deionized water (DIW), 12 mM sodium fluoride solution (NaF) and 150 mM calcium lactate pentahydrate solution followed by the sodium fluoride solution (CaL + NaF) – and salivary flow at 2 levels – normal and low – in an erosion-remineralization-abrasion cycling model using bovine enamel and dentin specimens (n = 8 per group for each substrate). The response variables were surface loss (micrometers), KOH-soluble fluoride and structurally bound fluoride (micrograms per square centimeter) measured at the end of the cycling phase, for each of the dental substrates independently. All the treatments and analyses were performed in random sequence and under blind conditions.

Specimen Preparation

Enamel and dentin slabs (3 mm width × 3 mm length × 2 mm thickness) were cut from bovine incisors using a microtome (Isomet, Buehler, Lake Bluff, Ill., USA). The specimens were embedded in acrylic resin (Varidur, Buehler), and the resulting blocks (10 × 10 × 8 mm) were ground flat and polished with water-cooled abrasive disks (500-, 1,200-, 2,400- and 4,000-grit SiC papers; MD-Fuga, Struers Inc., Cleveland, Ohio, USA) and polishing cloth with diamond suspension (1 μm; Struers Inc.). After the polishing procedures, specimens were sonicated in neutral detergent solution and rinsed with DIW. Specimens with cracks or any structural defects were discarded. Adhesive unplasticized polyvinyl chloride tapes were placed on either side of the polished surface of the selected specimens, leaving an area of 3 × 1 mm^2 exposed to subsequent testing. The specimens were then randomly assigned to the 6 experimental groups (n = 8).

Erosive Challenge and Remineralization

The daily cycling sequence is shown in figure 1. The erosive challenge was performed with a 0.3% citric acid solution (pH adjusted to 3.8 with 1 M KOH solution). A multichannel peristaltic pump (Masterflex LS, Cole Palmer, Ill., USA) was used. The specimens were individually placed in custom-made acrylic devices (4 specimens/device), constructed based on Wiegand et al. [2008], having their polished enamel or dentin surfaces facing a closed chamber of approximately 41.3 μl capacity (7.1 mm in diameter and approx. 1 mm in height). Each chamber was connected to 1 inlet and 1 outlet flow tubes. The inlet tube was connected to the pump through a 2-way tubing system, which included a second free-end tube to allow the elimination of the air bubbles during the experimental procedures. Before the first acid challenge of the day, DIW was rinsed through the chambers to remove the air through the tube with the free end. For this procedure, the tube connected to the pump was closed using a switch valve. Once the water had filled the chamber, the free-end tube was closed, and the tube connected to the pump was opened. Citric acid was then injected into the chambers at a rate of 0.6 ml/min for 2 min, at room temperature.

After the erosive challenge, the device was connected to another pump for remineralization. During this procedure, all the tubes from the system were closed to avoid the entrance of air into the chamber. After connecting the inlet tubes to the pump, a flush was needed to eliminate the air bubbles of the system. For the flush, the tube between the pump and the chambers remained closed and the air bubbles were released by the free-end tubes. Then, the free-end tubes were closed and the connection to the pump opened, to allow the fluid flow. Artificial saliva (1.5 mM $CaCl_2 \cdot 2H_2O$; 0.9 mM KH_2PO_4; 130 mM KCl; 20 mM of HEPES; pH adjusted to 7.0 with 1 M KOH solution) [ten Cate et al., 2008] was then injected into the chambers for 1 h, at a flow rate of either 0.5 ml/min, simulating a normal unstimulated flow rate [Navazesh and Christensen,

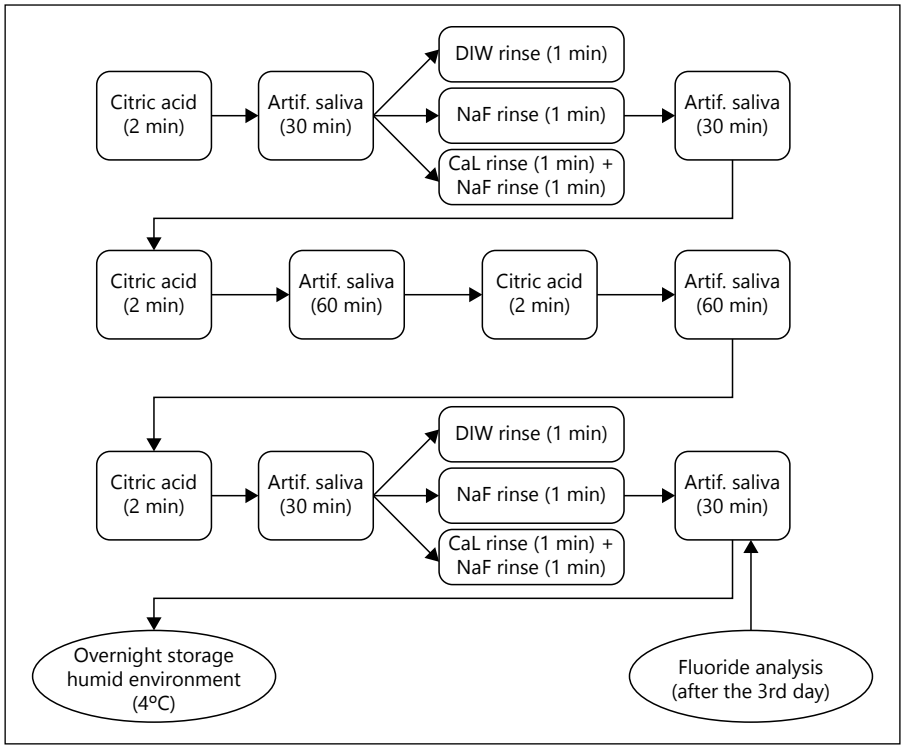

Fig. 1. Daily cycling sequence.

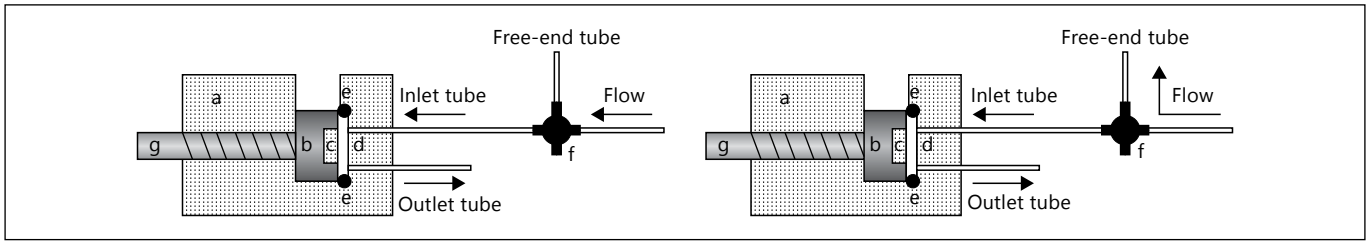

Fig. 2. Representation of the saliva vertical flow chamber (a). The block (b) containing the embedded enamel specimen (c) is positioned into the acrylic device, facing the chamber (d), which is sealed with a rubber O ring (e). In the left figure, the fluid is flowing towards the chamber and the free-end tube is closed using a switch valve (f). In the right figure, the inlet tube was closed turning the valve (f) to eliminate bubbles through the free-end tube. A screw was used to hold the block in position (g).

1982], or 0.05 ml/min, simulating a low unstimulated flow rate [Dawes, 2008].

The exposure of the specimens to the rinse solutions was performed twice a day by the free-end tube with the aid of a hypodermic syringe. In between the first and the last remineralization periods (30 min after the acid challenge), the chambers were rinsed with 20 ml of the following experimental solutions: DIW (negative control) for 1 min, NaF (12 mM sodium fluoride, pH 6.38) for 1 min, CaL + NaF (150 mM calcium lactate pentahydrate solution, pH 6.9) for 1 min immediately followed by the NaF [Vogel et al., 2008b]. For this procedure, the tube connected to the pump was closed. After rinsing, artificial saliva was again injected into the chambers for the completion of the 60 min. All solutions were used at room temperature (21 ± 2°C).

At the end of the day, specimens were removed from the chambers, rinsed with DIW, dried and stored overnight in a closed container with an approximately 100% relative humidity at 4°C. Figure 2 shows a representation of the chamber and the mechanism of fluid flow in the 2-way system.

Surface Loss Assessment

After completing 3 days of cycling, the tapes were removed and specimens were left to dry for 10 min before analysis. An area 2 mm long (X) × 1 mm wide (Y) was scanned with an optical profilometer (Proscan 2000, Scantron, Venture Way, Taunton, UK), covering both the treated and reference surfaces. The step size was set at 0.01 mm and the number of steps at 200 in the X axle, and at 0.1 mm and 10, in the Y axle. The depth of the treated area was

Table 1. Geometric means (antilog SE in parentheses) of the KOH-soluble fluoride (μg/cm^2) and structurally bound fluoride (μg/cm^2) for the experimental groups

Rinses	Enamel						Dentin					
	KOH-soluble F			struct. bound F			KOH-soluble F			struct. bound F		
	LF	NF		LF	NF		LF	NF		LF	NF	
DIW	1.75 (1.07)	1.00 (1.15)	a	0.33 (1.15)	0.16 (1.11)	a	0.80 (1.16)	0.53 (1.15)	a	0.16 (1.14)	0.14 (1.15)	a
NaF	2.84 (1.16)	1.70 (1.10)	b	0.93 (1.07)	0.71 (1.13)	b	8.00 (1.23)	3.60 (1.15)	b	0.82 (1.12)	0.87 (1.11)	b
CaL + NaF	23.92 (1.18)	8.51 (1.32)	c	1.51 (1.09)	0.99 (1.21)	c	15.63 (1.06)	6.16 (1.14)	c	1.25 (1.13)	1.02 (1.19)	b
	A	B		A	B		A	B		A	A	

Different capital letters denote a significant difference between the salivary flows ($p < 0.01$). Different lowercase letters imply a significant difference ($p < 0.01$) between the groups within the flow rate, in columns.

calculated based on the subtraction of the average height of the test area from the average height of the two reference surfaces by using a 3-point height tool, in dedicated software (Proscan Application software v. 2.0.17).

KOH-Soluble Fluoride Determination

Unplasticized polyvinyl chloride tapes were replaced on the polished surface of the specimens, leaving the lesion area (3 × 1 mm) exposed. The determination of KOH-soluble fluoride from enamel and dentin surfaces was performed based on a previously described method [Caslavska et al., 1975]. The specimens were individually stored in plastic containers with 0.5 ml of 1.0 M KOH solution, under gentle agitation at room temperature for 24 h. After this period, the specimens were rinsed with DIW, and a sample of the solution (0.25 ml) was transferred to a plastic vial and neutralized with 0.25 ml of 1 M $HClO_4$. Then, 0.5 ml of TISAB II buffer was added to the tube. The fluoride content was determined by comparison to a similarly prepared standard curve using an ion-selective electrode (Orion EA940, Thermo Electron Corporation, Beverly, Mass., USA).

Structurally Bound Fluoride Uptake

This analysis was performed immediately after the KOH-soluble fluoride determination. Specimens were immersed in 0.5 ml of 1 M $HClO_4$ for 15 s, under agitation. A sample (0.25 ml) of the solution was transferred to a plastic vial and neutralized with 0.25 ml of 1 M NaOH. Then, 0.5 ml of TISAB II buffer was added to the tube. The fluoride content was determined as described above.

For both analyses, the concentration of fluoride was expressed in micrograms per square centimeter.

Statistical Analysis

Surface loss data was tested for normal distribution and homoscedasticity with Shapiro-Wilk and Hartley tests, respectively. Since both assumptions were satisfied, 2-way ANOVA and Tukey tests were carried out for comparisons among groups. The results of the fluoride analyses were transformed by $\log_{10}$, to satisfy the assumptions of the ANOVA. The significance level was set at 5%. The software SigmaPlot 12 (Systat Software Inc., San Jose, Calif., USA) was used for the calculations.

Results

Fluoride Analyses

The results of the fluoride analyses are shown in table 1. No significant interaction was observed between rinse treatment and salivary flow ($p > 0.05$), for the KOH-soluble fluoride and for the structurally bound fluoride analysis, in both substrates. For enamel, low saliva flow showed higher fluoride concentrations than normal flow ($p < 0.01$), in both analyses. Similarly, CaL + NaF presented the highest F concentrations followed by NaF and DIW (all different at $p < 0.01$), in both F analyses. For KOH-soluble F in dentin, low flow presented more fluoride than the normal flow ($p < 0.01$) and the CaL + NaF rinses showed the highest fluoride values, followed by the F rinse and DIW (all different at $p < 0.01$). In the structurally bound fluoride analysis, no significant difference was observed between salivary flows ($p = 0.636$). Within rinses, CaL + NaF and NaF presented higher F concentrations than DIW ($p < 0.01$), but did not differ statistically significantly from each other ($p = 0.103$).

Surface Loss

Means (and SD) of enamel and root dentin surface loss (in micrometers) for all groups are presented in figure 3. For both substrates, a significant interaction was observed between the two studied experimental factors ($p < 0.01$). Within the factor salivary flow, significantly more surface loss was observed for low flow compared to normal flow ($p < 0.01$), for treatments DIW and NaF (asterisk in fig. 3). No difference on erosion was observed between the different flow rates for CaL + NaF ($p = 0.164$ for enamel and $p = 0.293$ for dentin). Within the factor rinse treatment and for both salivary flow conditions, DIW exhibited signifi-

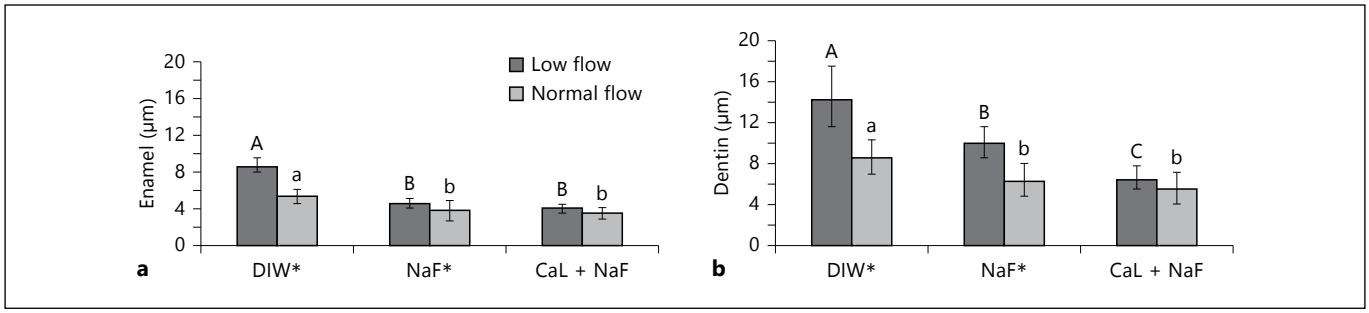

Fig. 3. Means (±SD) of enamel surface (**a**) and dentin loss (**b**) in micrometers for the experimental groups. Different lowercase superscript letters imply a significant difference between the salivary flow rates within the rinses. Different capital superscript letters denote a significant difference among the groups for the rinse factor. Asterisks indicate differences in the comparison between levels (low vs. normal) within each treatment rinse.

cantly more enamel surface loss than NaF and CaL + NaF rinses ($p < 0.01$), which did not differ from each other. For dentin, under conditions of normal flow, the surface loss was higher in the DIW group ($p < 0.01$) compared to the NaF and the CaL + NaF groups, with no difference between them. At low flow, all treatments were significantly different ($p < 0.01$), with CaL + NaF presenting the lowest values of surface loss followed by NaF and DIW.

Discussion

The results of this study showed a significant protective effect of a sodium fluoride rinse against erosion on enamel and root dentin, regardless of the salivary flow rate tested. This corroborates previous in vitro studies on the benefit of different forms of topical sodium fluoride [Ganss et al., 2001; Parkinson et al., 2010; White et al., 1994, 2012]. Additionally, we hypothesized that a prerinse with calcium lactate would improve the availability of fluoride, enhancing its protective effect on erosion. Although the hypothesis was accepted for fluoride analyses (except for structurally bound fluoride on dentin), the additional protective effect was not observed under simulated normal unstimulated salivary flow conditions, since no significant differences in surface wear were observed between NaF and CaL + NaF groups, on either enamel or dentin substrates. This lack of benefit contrasts with what has been suggested in a previous study on dental caries [Vogel et al., 2008b] and is not completely unexpected, considering the proposed mechanism of action of the CaL prerinse. Under cariogenic conditions, the beneficial increase in fluoride retention occurs mainly in the biofilm [Pessan et al., 2006], which does not play a role in the development of dental erosion. In fact, dental biofilm can protect the tooth surface against erosion [Honorio et al., 2010]. Therefore, since we simulated an erosion model and not caries, any protective effect from calcium and fluoride in the studied model would be due to their retention on the experimental dental surfaces. In intraoral conditions, additional retention sites such as oral soft tissues [Zero et al., 1992b] and possibly the dental pellicle [White et al., 2012] are also available and can potentially improve this calcium action, although it has yet to be proven. This may explain why CaL prerinse protection was observed in a preliminary in situ study with enamel under normal flow conditions [Turssi et al., 2012].

Interestingly, when low salivary flow conditions were adopted, a significant increase in protection was observed for the CaL + NaF rinse treatments, but only on root dentin. Benefits on enamel were not significant. Nevertheless, when the salivary flow conditions were compared, no significant differences were observed for the groups treated with CaL + NaF, as one could expect. This suggests that CaL + NaF treatment compensated for the more erosive condition imposed by the low flow, for both enamel and dentin substrates.

In the present study, neither the changes on the dental structure nor the mineral composition of the deposits on the tooth surface were directly analyzed, limiting the interpretation of the mechanism of action of calcium and fluoride. However, the analysis of alkali-soluble and structurally bound fluoride can provide some support for the hypothesis explaining the dental surface loss results mentioned above. It is noteworthy that the fluoride values for enamel and dentin should not be directly compared, since the substrate surfaces are different, as described below. For enamel, both fluoride forms were significantly

higher when CaL was used before the F rinse. As fluoride can adsorb to calcium sites on the crystal lattice or on the hydroxyapatite hydration layer, the calcium prerinse may have worked as labeled binding site for nonspecific adsorbed fluoride [Rolla and Bowen, 1978]. Since the combination of low NaF concentration with neutral pH and short time do not result in CaF_2 precipitate formation [Petzold, 2001], it is suggested that at the fluoride rinse concentration tested, the main mechanism of enamel erosion protection would be related to the adsorption of fluoride ions onto the enamel surface. These ions would then be released to the teeth surroundings by the time of the acid challenge reducing, therefore, the demineralization rate. Alternatively, as can be seen in the fluoride analysis results (table 1), supplying additional Ca to the substrate considerably increases KOH-extractable F. In fact, it was previously demonstrated that the pretreatment with calcium increases the formation of calcium fluoride-like deposits on the enamel surface [Saxegaard and Rolla, 1988]. The amount of KOH-soluble fluoride increased from approximately 5 to 9 times in normal and low flow conditions on enamel specimens treated with CaL + NaF. However, despite this huge increase, there were no significant reductions in enamel loss, probably because these CaF_2-like precipitates are readily dissolved at low pH, limiting the fluoride action on enamel [Ganss et al., 2004a]. Nevertheless, this protective effect was observed when low salivary flow was simulated, probably because it helped keeping fluoride ions available in the testing environment for longer periods, compared to the normal salivary flow condition. In the experimental model used, the rinse solutions were injected into the testing chambers via a free-end tube of the system; consequently, the clearance of the rinses was slower in the low salivary flow simulation, reproducing what occurs clinically when topical fluoride agents are applied [Zero et al., 1992a].

It has to be noted that, in order to detect the amount of fluoride resulting from the rinse applications, fluoride analyses were performed at the end of the experiment, after the last treatment rinses and exposure to the remineralizing solution. This could only be done at this point, as these analyses are of destructive nature. Different results could have been found had these analyses been performed after the acid challenge, potentially showing loosely bound fluoride levels that would better reflect the surface loss results.

The differences in surface loss between enamel and dentin when treated with Ca + F and under low salivary flow seem to be related to their morphologies and physicochemical properties, as the presence of dentinal tubules and demineralized organic matrix may provide additional retention sites for fluoride and calcium, compared to the relatively smooth and uniform enamel surface. CaF_2-like deposits can be retained mainly in peritubular and intertubular dentine, but also in intratubular regions [Laufer et al., 1981]. The demineralized dentin presents a wide reactive surface area, facilitating the fluoride uptake by surface absorption and/or ionic exchange with surface hydroxyl ions [Spinelli et al., 1971]. Additionally, the exposed organic layer further increases the dentin surface area and the diffusion pathways, enhancing the amount of KOH-soluble fluoride [Buchalla et al., 2007] and also of calcium, potentially. Higher amounts of fluoride, especially due to calcium prerinse, may increase its action stabilizing the mineral structure [Rolla and Bowen, 1978], providing higher protection against dentin erosion. It was shown that this surface layer of demineralized organic matrix is essential for the fluoride protective action against erosion [Ganss et al., 2004b]. Nonetheless, it has to be pointed out that in clinical conditions, this organic layer may not be retained, as a result of enzymatic [Ganss et al., 2004b; Schlueter et al., 2010] and/or mechanical degradation. Hence, caution should be taken when extrapolating the results of this study to the clinical situation.

The in vitro experimental model used was able to reproduce the differences between the tested salivary flow conditions, possibly simulating the higher risk for dental erosion attributed to the hyposalivatory population. Additional relevant aspects not considered were the reproduction of toothbrushing abrasion and of acquired dental pellicle, as both these factors have previously been shown to be important on the development of dental erosion [Hara et al., 2006; Martins et al., 2012]. These aspects should be further tested with clinically relevant in situ models.

The results of the present study show that the use of a CaL prerinse may be potentially interesting for high-risk populations, in particular the hyposalivatory one with exposed root surfaces, as the lack of saliva may potentially increase the retention of Ca and F in the oral environment, enhancing protection against dental erosion. The concentrations of the rinse solutions used in this study were based on previous investigations, which have shown that rinsing with a highly concentrated CaL solution before the daily sodium fluoride rinse (228 ppm F, as NaF) increases fluoride retention in saliva [Vogel et al., 2006, 2008a]. In practical terms, the subsequent use of two rinse solutions could be a potential limiting factor, as it requires one additional step for the patient. Therefore, future studies testing more practical associations are war-

ranted. For instance, the association of a CaL rinse with fluoride toothpastes may be considered, since dentifrices are the most widespread vehicles of fluoride delivery but seem to present limited benefit on erosion prevention [Magalhaes et al., 2007].

In conclusion, the sodium fluoride rinse was able to reduce erosion progression, which was significantly higher under low salivary flow rate conditions. The calcium lactate prerinse increased fluoride availability on dental surfaces and enhanced the fluoride protection against dentin erosion in hyposalivatory conditions.

Disclosure Statement

The authors declare that they have no conflict of interests.

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